A Guide to Bacterial Culture Identification And Results Interpretation

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ABSTRACT

Purpose: To provide a guide to interpreting bacterial culture results.

Methods: Studies were identified via a PubMed literature search (from 1966 to January 2018). Search terms included microbial sensitivity tests, microbial drug resistance, and anti-infective agents/pharmacology. Articles were included if they were published in English. References within identified articles were also reviewed.

Results: This paper reviewed core concepts of interpreting bacterial culture results, including timing of cultures, common culture sites, potential for contamination, interpreting the Gram stain, role of rapid diagnostic tests, conventional antibiotic susceptibility testing, and automated testing.

Conclusion: This guide can assist pharmacists in their role as integral members of the antimicrobial stewardship team in an effort to improve patient care.

Keywords: microbial sensitivity tests, microbial drug resistance, anti-infective agents/pharmacology, pharmacokinetics

INTRODUCTION

Currently, there is a shortage of pharmacists trained in infectious diseases to fill antimicrobial stewardship positions across the United States. This means that pharmacists in a variety of other positions must take on these roles in order to improve appropriate antibiotic prescribing.[1] In hospital settings, studies suggest that approximately 50% of patients receive at least one antibiotic during their inpatient stay, 30% of which includes broad-spectrum antibiotics.[2–5] The inappropriate use of antibiotics has been reported to be as high as 50%, although estimates can vary by institution and how "appropriate" is defined.[6–9] Inappropriate use may lead to increased adverse effects, secondary infections, drug interactions, additional costs, prolonged lengths of stay, and hospital readmissions. Furthermore, bacterial resistance may develop, which can lead to treatment failure.[9,10] This paper covers the timing of cultures, common culture sites, interpreting the Gram stain, the role of rapid diagnostic tests, conventional antibiotic susceptibility testing, and automated testing. Throughout the article, we use the term *antibiotic* instead of *antimicrobial*, as we did not cover the testing of viruses or fungi.

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OBTAINING CULTURES

Medical professionals should obtain cultures quickly to guide therapy, simultaneously taking into consideration the importance of prompt antibiotic administration.[11] The 2016 Surviving Sepsis Campaign guidelines recommend that antibiotics be administered within one hour of sepsis diagnosis and that cultures should not delay antibiotic administration for more than 45 minutes.[12] In septic patients, the administration of appropriate antibiotics within one hour can reduce mortality.[13] Obtaining cultures before antibiotic administration can assist clinicians in identifying the offending organism, allowing potential de-escalation through proper treatment. If cultures are drawn post-antibiotic administration, there may be a decrease in the blood-culture yield, which can increase the cost and length of stay for the patient.[14–16]

Cultures may be obtained from sites that are either colonized with bacteria or sterile. Those colonized with bacteria increase the risk of contamination from normal flora and may lead to false results. Sites that are typically thought of as sterile include cerebral spinal fluid, blood, and pericardial fluid. Sites that are well known for contamination include sputum and nasal passages.[17] Poor culture-collection technique may also increase the risk of contamination. Considerations for interpreting results for blood, respiratory, urine, skin and soft tissue, bone and joint, cerebral spinal fluid, and stool cultures are provided below.

Blood Cultures

When evaluating blood cultures, it is important to differentiate between bacteremia and contamination. Contamination occurs when bacteria from an outside source are introduced into a collected sample.[18] For example, normal skin bacteria may be introduced when a single needle venipuncture is performed. Bacteria may also be present in medical devices, such as central venous catheters. Central venous catheters are associated with higher rates of contamination than venipuncture. Rates of contaminant identification are increasing, as newer methods of testing improve the identification of bacteria, even in small amounts.

When evaluating potential contamination, the clinician should assess the patient's clinical presentation and determine whether he or she shows signs and symptoms of bacteremia. For example, if a patient was hypotensive, tachycardic, and febrile, this would suggest the presence of bacteremia. In addition, it is vital to draw at least two samples from separate sites of the body. The second site is often a central venous catheter, if present. If

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one of the two cultures grows an organism that is considered a likely contaminant, repeat cultures are necessary. Conversely, if multiple samples grow the same organism, true bacteremia is usually the result.[19,20] Only one positive culture is needed to suggest true infection in patients with gram-negative bacteria.[21] Gram-positive bacteria, especially *Staphylococcus epidermidis* and *Corynebacterium* species, are more likely to be contaminants.[22] One study evaluating 500 blood cultures found that *Staphylococcus epidermidis* and *Corynebacterium* species were contaminants 94% and 79% of the time, respectively. However, in populations such as immunosuppressed patients, these two bacteria could represent true infection. Gram-positive bacteria that are associated with true infection include *Staphylococcus aureus* and *Streptococcus pneumoniae*. Repeat cultures should be drawn to ensure the resolution of bacteremia.

Respiratory Cultures

To diagnose pneumonia, many clinicians order sputum cultures, endotracheal aspirates, and, less frequently, broncho alveolar lavage. These are performed in addition to the evaluation of signs and symptoms (cough, fever, sputum production, pleuritic chest pain) and chest radiography.[23] Sputum cultures can be difficult to interpret because the upper respiratory tract is colonized with bacteria, in contrast to the lower respiratory tract, which is typically sterile.[24] Reports of sensitivity and specificity of sputum cultures vary widely.[23] If a sputum culture is obtained, then one needs to note the presence of epithelial cells, which can represent culture contamination. Sputum cultures should have less than 10 epithelial cells per low-powered field. In certain patient populations, such as those with chronic obstructive pulmonary disease (COPD) who are experiencing an exacerbation, sputum cultures should be avoided.[25] Instead of using such cultures to determine whether an infection is present, the 2017 Global Initiative for Chronic Obstructive Lung Disease guidelines recommend evaluating patient symptoms, such as sputum production, purulence, and dyspnea. For patients who are mechanically ventilated, cultures are typically obtained through endotracheal aspiration or bronchoalveolar lavage. Endotracheal aspiration has a lower specificity than bronchoalveolar lavage for the diagnosis of pneumonia, although it has fewer complications and a lower cost.[26] This lower specificity can result in higher antibiotic utilization. Guidelines differ on the preference for endotracheal aspiration or bronchoalveolar lavage, as the choice is based on antibiotic de-escalation or the costs and complications of these techniques.[27,28] Clinicians should refer to their institution's standards when recommending endotracheal aspiration or bronchoalveolar lavage.

Urine Cultures

Urine cultures should only be drawn when infection is suspected, usually in the presence of patient-reported symptoms.[29] Typical urinary infection symptoms include dysuria, frequency, and urgency, although patients with dementia may present with non-specific signs such as fatigue and mental status changes.[30] In patients with urinary catheters, symptoms are more generalized and can include fever, weakness, and altered mental status.[31] Screening for asymptomatic bacteriuria should only be done in pregnant patients or in patients undergoing urologic procedures.[29] When evaluating a patient with a potential urinary tract infection (UTI), the urine culture should be evaluated along with symptoms and the urinalysis. Ten or more leukocytes per microliter in the urinalysis is associated with a diagnosis of UTI, but this should not be the sole criterion used for diagnosis. Cultures in patients without urinary catheters are best collected midstream to avoid contamination. In the urine culture, the number of colony-forming units (CFUs) per ml is an estimate of the number of bacteria in the sample. More than 10^3 CFUs per ml in the urine culture is associated with infection. However, true infection may occur in patients with lower CFUs in the presence of symptoms and urinary leukocytes.[32] Patients with catheter-associated UTI also have lower CFUs. Conversely, higher CFU counts can occur in the presence of contaminants, and after an incorrectly collected specimen. Bacteria typically not seen in UTIs may be caused by contamination, unless the patient has risk factors for other types of bacteria and the sample is appropriately collected. Common bacteria in uncomplicated UTIs include *Escherichia coli*, *Klebsiella* species, *Proteus* species, and *Staphylococcus saprophyticus*.

Skin and Soft Tissue Infections

The Infectious Diseases Society of America (IDSA) recommends obtaining cultures for abscesses and carbuncles, although they mention empiric treatment without culture as being reasonable.[33] For cellulitis, the IDSA recommends only obtaining blood cultures if the patient has specific factors. These factors include: immunosuppression, malignancy, animal bites, and/or immersion injuries.[34] Skin-surface cultures should only be obtained in the setting of purulent drainage. Obtaining skin cultures in infections without drainage often leads to identification of polymicrobial organisms that are not the cause of infection, leading to overly broad treatment.[35] As with UTIs, the patient's history and examination are important for making the diagnosis. Patients with purulent drainage are more likely to be infected with *S. aureus,* and patients with non-purulent cellulitis are more likely to be infected with group A streptococci.[36]

Bone and Joint Infections

Bacterial joint and bone infections can occur when bacteria are introduced from blood, prostheses, trauma, or the extension of a skin infection. Knowing the source can help predict which bacteria may be causative. Typical symptoms of joint infections include fever, painful and swollen joints, tenderness, and decreased range of motion.[37] Patients with joint prostheses or recent joint surgery (usually < 3 months) are more likely to have joint infections.[38] Joint infections are diagnosed through synovial fluid cultures, where the fluid is drawn in surgery or through aspiration. Elevated white blood cell (WBC) counts in the synovial fluid, especially when the count exceeds 50,000 cells/mm^3, can help rule in (90% specificity) but not rule out (56% sensitivity) joint infections.[39] The Gram stain also has a low sensitivity (45–71%) for the diagnosis of joint infections. Lastly, the evaluation of protein or glucose in the synovial fluid does not help with diagnosis. Patients with osteomyelitis typically present with symptoms of fever, lethargy, inflammation, erythema, and swelling.[40] Bone biopsy cultures and radiographs play a major role in diagnosing osteomyelitis, along

with identifying the offending organism. Blood cultures can also be drawn to determine the organism in hematogenous osteomyelitis. Lastly, patients typically have elevated WBC counts, an elevated erythrocyte sedimentation rate, and elevated C-reactive protein levels, although these cannot be used in diagnosis without a culture.[41]

Cerebrospinal Fluid Cultures

Cerebrospinal fluid (CSF) cultures play a major role in the diagnosis of meningitis.[42] CSF cultures associated with untreated bacterial meningitis typically show an elevated WBC count (> 1,000 cells/mm^3), although lower WBC counts may be seen. Elevations in WBC count can also occur if the lumbar puncture is traumatic. A general rule is that every 1,000 red blood cells in the CSF increases the WBC CSF count by one.[43] Neutrophils are usually > 80%, although in 10% of patients, lymphocytes > 50% can be seen. Also, in bacterial meningitis, CSF cultures frequently have a high protein count (> 0.9 g/L) as protein has increased penetration into the CSF when meningeal inflammation is present.[44] During bacterial meningitis, glucose is utilized by bacteria; therefore, a low CSF glucose count (< 40 mg/dl) or ratio between the CSF glucose and serum glucose (≤ 40%) can be seen. The opening pressure on lumbar puncture may also be elevated above 200 mm H_2O.[43] Gram stain is useful in the diagnosis of patients with bacterial meningitis, especially with a larger bacterial burden. In some cases, specificity of the Gram stain has been reported to be as high as 97%. Lastly, polymerase chain reaction (PCR) has high sensitivity and specificity, and could be used to rule out bacterial meningitis if the CSF Gram stain and culture are negative.[45]

Stool Cultures

Stool cultures may be considered in patients who present with persistent diarrhea or bloody diarrhea, or who have recently traveled to areas with poor public sanitation systems. Obtaining one stool culture is sufficient and can detect the pathogen 87–94% of the time.[46] Laboratories routinely screen for bacteria such as *Salmonella, Shigella, E. coli, and Campylobacter* in stool cultures. In addition, *Clostridium difficile* testing may be considered in patients with risk factors for the infection, such as having taken antibiotics in the last two months, having more than two unformed bowel movements in a 24-hour period, increases in WBC count, worsening renal function, and decreases in albumin.[47] Several tests are available for the detection of *C. difficile*. Most commonly, these tests include toxin A and B enzyme immunoassay, glutamate dehydrogenase (GDH) immunoassay, and molecular assay. Most laboratories perform the toxin A and B immunoassay first, usually concomitantly with the GDH assay. Using these two tests together has a high specificity (97% or greater) but the sensitivity of the tests is lower (41–92%).[48,49] If both the toxin A and B enzyme immunoassay and the GDH are positive, then *C. difficile* is likely. If one is positive and the other is negative, further testing with molecular assays should occur, as this can improve sensitivity and specificity. Testing after resolution is not helpful as stool cultures will remain positive for weeks after infection.

EARLY BACTERIAL IDENTIFICATION

It is essential to carry out antibiotic-susceptibility testing because it guides clinicians in selecting an appropriate treatment regimen for an infection. Different approaches to early bacterial identification include Gram staining, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF), PCR, nanoparticle probe technology, and peptide nucleic acid fluorescence in situ hybridization (PNA-FISH).

Gram Staining

Gram staining is a diagnostic test that gives an early indication of potential bacteria through visualization of the bacteria. The Gram stain helps to differentiate the organism, whether it is gram-positive or gram-negative.[50] Gram-positive bacteria appear purple in color and gram-negative bacteria appear pink. In addition, the shape, arrangement, and size of the organism can provide further information to help identify the organism. Common shapes seen on the Gram stain include cocci, which resemble spheres; bacilli, which resemble rods; or coccobacilli, which are a combination of the two. Cocci can be arranged in patterns, such as clusters or chains; for example, Staphylococcus species appear as gram-positive cocci in clusters. Size may help a microbiologist differentiate bacilli, although this is not often reported to the clinician.

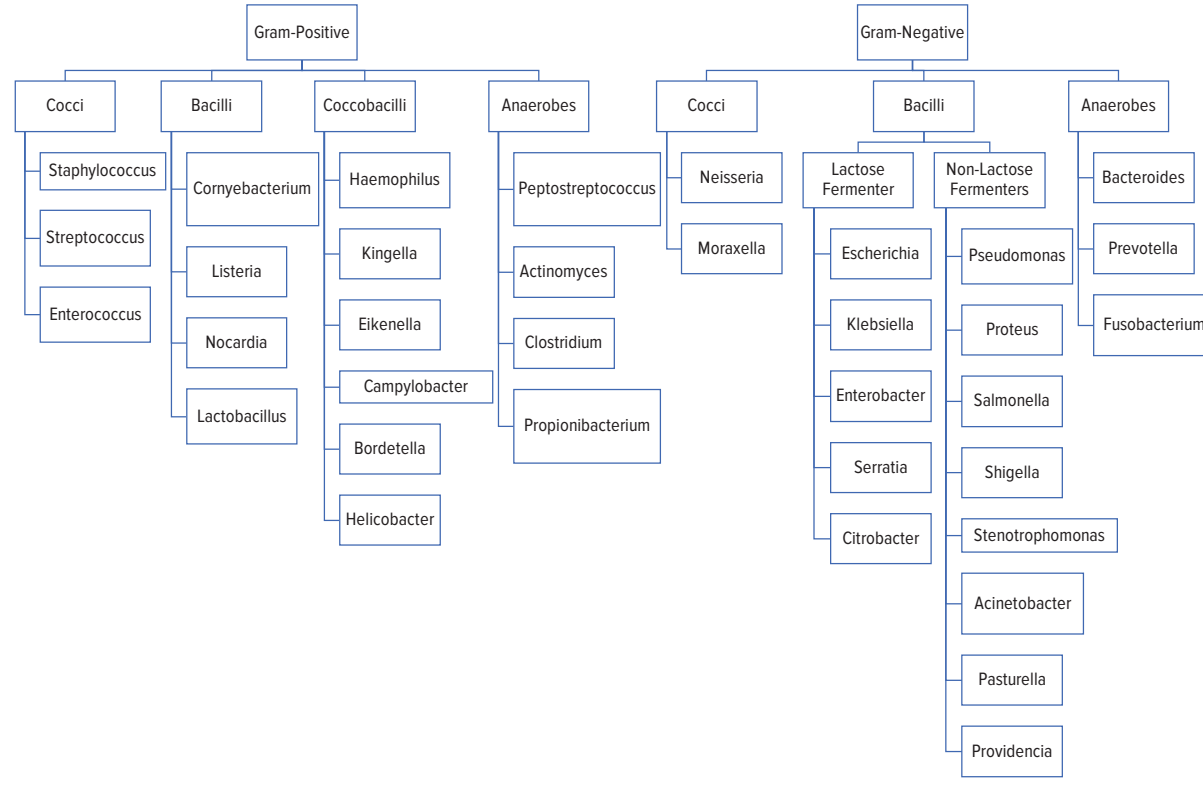
To perform a Gram stain, the technician applies bacteria to a slide then passes it over a flame to ensure the bacteria stay on the slide. Next, crystal violet dye is applied, which stains all of the bacteria purple. Iodine is then applied, which helps the dye bind to the peptidoglycan layer of the cell wall, and this is followed by acetone, which washes away the dye. The purple dye stays on gram-positive bacteria as a result of the strong bond between the bacteria and a thick peptidoglycan layer, but it washes away from gram-negative bacteria, which has a thin peptidoglycan layer. Lastly, a dye such as safranin is applied, which stains gram-negative bacteria pink.[51] Other tests may be performed to further differentiate bacteria. MacConkey agar plates can be used to differentiate lactose-fermenting and nonlactose-fermenting gram-negative bacteria. The coagulase test can be used to differentiate between *Staphylococcus aureus* and other Staphylococcus species. If the coagulase test is positive, the bacteria is likely *Staphylococcus aureus*; if it is negative, the bacteria is likely *Staphylococcus epidermis* or *Staphylococcus saprophyticus.*

Understanding the patterns of preliminary growth of organisms can guide clinicians in selecting the appropriate antibiotic. A flowchart is provided in Figure 1 to assist with preliminary bacterial identification. However, sensitivity and specificity of the Gram stain can vary widely; therefore, further microbiologic identification is performed prior to de-escalation.[52,53] Nonetheless, Gram stains are a useful tool in guiding empiric therapy prior to obtaining final culture results.

Rapid Diagnostic Tests

Rapid diagnostic tests can assist in faster identification and de-escalation of antibiotic therapy. Pharmacists have played a critical role in providing recommendations to providers based on these tests.[54,55] These rapid diagnostic systems include MALDI-TOF, PCR, PNA-FISH, and nanoparticle probe technology. MALDI-TOF can be explained by deconstructing the acronym. To perform MALDI-TOF, a sample is grown on an

Figure 1 Classification of Common Gram-Positive and Gram-Negative Bacteria



agar plate and placed onto a MALDI plate, whereupon a matrix is added. The **MA**trix helps transform a **L**aser into heat, which causes the sample to **D**esorb from the plate and form **I**onized molecules. The molecules turn into gas, which then fly into the **T**ime-**O**f-**F**light tube based on their size and charge. Molecules with a smaller size-to-charge ratio travel faster, which creates a spectrum based on the size and charge of the molecules. This spectrum is matched up to the spectra libraries for a known organism, such as *Escherichia coli*. The spectra also correlate to a score value on the MALDI-TOF machine, which indicates the probability of correctly identifying the genus and/or species being tested.[56] The main advantage of MALDI-TOF is the rapid (less than five hours) identification of the bacteria. However, the test does not currently provide susceptibility information and cannot detect heteroresistance. Heteroresistance occurs when a subpopulation of bacterial cells displays higher antibiotic resistance than the remainder of the culture.[57]

An alternate, rapid identification method is PCR, which works by making multiple copies of DNA segments that are used to identify bacteria. To perform PCR, a DNA segment, nucleotides, polymerase, and a primer are needed.[58] The DNA is heated, which causes it to split into two single strands. These strands are primed, and then the polymerase makes new DNA strands using the nucleotides. The process is repeated to make multiple copies of DNA segments that can be analyzed to identify the microbe. The advantages of PCR include rapid identification (less than one hour) of bacteria and the ability to detect bacteria while the patient is on antibiotic therapy. But the technique also has some limitations, as PCR may detect bacteria that are not viable after a patient has been treated for an infection.[59] Furthermore, accuracy can decrease in polymicrobial infections. Multiplex PCR, an extension of PCR, addresses this issue by using multiple primers, which allows for the identification of multiple bacteria. This also allows for the detection of antibiotic resistance genes.

Nanoparticle-probe technology also uses DNA to help identify bacteria. This test, which uses nanogrids (slides covered in DNA) to bind to gram-positive or gram-negative DNA, is only used in patients who have positive blood cultures.[60] When binding occurs, gold nanoparticles are attached, then a silver solution is washed over the slide and the solution binds to the gold. A light passed over the slides allows visualization of the silver by scattering the light into a camera. Advantages of this test include rapid identification (less than three hours) of a large number of bacteria and resistance mechanisms. Its limitations include the requirement of a positive blood culture and the fact that not all potential pathogens are identifiable.

Unlike PCR and nanoprobe-particle technology, which identify bacteria by the detection of DNA, PNA-FISH identifies bacteria using probes to identify ribosomal RNA. PNA-FISH can also be explained by deconstructing the acronym. In this procedure, **F**luorescent **p**eptide-**n**ucleic **a**cid (**PNA**) probes are applied to bacteria fixed to a slide.[61] These probes **H**ybridize (bind) to the ribosomal RNA and are viewed under a fluorescence microscope in real-time (*In Situ*). Advantages of PNA-FISH include rapid detection (less than one hour), the ability

to identify bacteria that are harder to grow with traditional methods (anaerobes, *Mycoplasma* species), and a decreased cost relative to PCR. The main disadvantage of PNA-FISH is that the investigator must anticipate what the potential bacteria are, as different bacteria have different kits. Knowing the Gram stain can help in selecting the appropriate kit. Thus, if lactose-positive gram-negative bacilli were growing, the technician would select the kit to be used with *E. Coli/P. Aeruginosa*. Also, PNA-FISH can only detect drug resistance if mutations occur with ribosomal RNA.

CONVENTIONAL BACTERIAL IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY TESTING

Conventional identification of bacteria consists of performing Gram stain followed by bacterial identification and antibiotic-susceptibility testing. The process from start to finish can take up to five days, which can delay time to antibiotic de-escalation.[59] Antibiotic susceptibility standards in the U.S. are determined by the Food and Drug Administration (FDA), which mostly refers to standards set by the Clinical and Laboratory Standards Institute (CLSI). Standards may vary in other countries, which can lead to discrepancies in the definition of antibiotic susceptibility; this has led to the creation of the National Antimicrobial Susceptibility Testing Committee for the USA (USCAST). This committee works with the European Union Committee for Antimicrobial Susceptibility Testing to normalize standards on an international level. The determination of antibiotic susceptibility uses quantitative data that is further split into qualitative categories, as seen in Table 1.

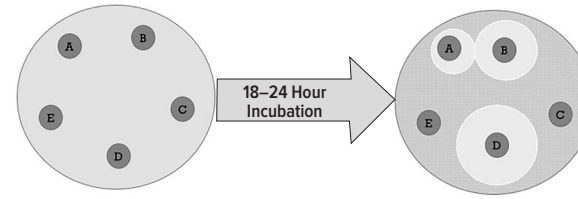
Quantitative testing is typically performed by identification of the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of antibiotic needed to inhibit the growth of an organism. The lowest MIC does not always correlate to the most effective treatment, as different antibiotics achieve different concentrations in different sites.[62] To determine qualitative categories, specific MIC values, also known as breakpoints, are used to classify bacteria as susceptible, intermediate, or resistant to a specific antibiotic. To determine breakpoints, the CLSI uses pharmacokinetic, pharmacodynamic, resistance mechanisms, and clinical data.

Table 1 Final Antibiotic Susceptibility Report for *Klebsiella Pneumoniae*

	MIC (mcg/mL)	Interpretation
Amikacin	≤ 2	S
Ampicillin	≥ 32	R
Ampicillin/Sulbactam	16	I
Cefazolin	≥ 64	R
Cefepime	2	R
Ceftriaxone	≥ 64	R
Ciprofloxacin	≥ 64	R
ESBL*	NEG	
Gentamicin	≥ 16	R
Imipenem	≤ 1	S
Piperacillin	≥ 128	R
Piperacillin/Tazobactam	1.5	S
Tigecycline	≤ 0.5	S
Tobramycin	8	I
Trimethoprim/Sulfamethoxazole	≥ 320	R

*ESBL= extended-spectrum beta-lactamases

Figure 2 Disk Diffusion (Kirby-Bauer Method)



Antibiotics are added to the plate after an organism is streaked onto the agar. After a period of incubation, a zone of inhibition can be seen that is used to determine susceptibility.

The CLSI defines as susceptible those isolates that are inhibited by typical achievable concentrations of an antibiotic when the dosage recommended to treat the infection site is used.[63] The susceptible dose-dependent category is determined when a dosing regimen is required that results in more drug exposure than that which is used in the susceptible category to achieve clinical efficacy. The intermediate category includes isolates that approach usually attainable levels in blood and tissue by an antibiotic, and response rates are lower than susceptible isolates. Lastly, the resistant category encompasses isolates that are not inhibited by the usual achievable concentrations of an antibiotic agent or for which resistance mechanisms are likely and clinical efficacy of antibiotics has not been reliably shown.

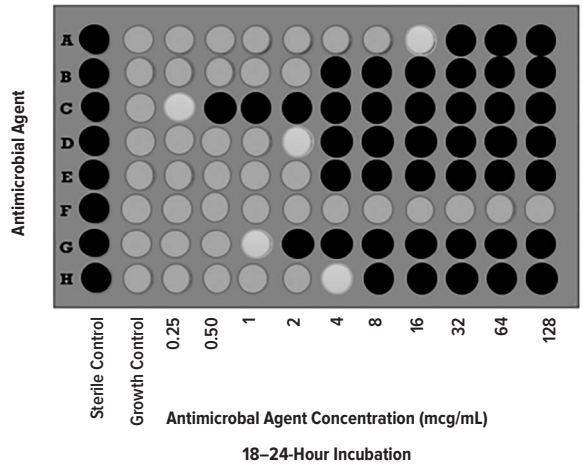
Diffusion Testing

The disk diffusion, or Kirby-Bauer, method, is a common test used to determine antibiotic susceptibility (see Figure 2). Diffusion testing works by placing an antibiotic disc onto an agar plate containing bacteria. The plate is incubated for up to 24 hours and during this time, the antibiotic diffuses throughout the plate, forming a concentration gradient surrounding the disc.[64] As the antibiotic concentration decreases, bacteria are more likely to grow. The diameter of the area displaying no growth is measured to determine susceptibility as per CLSI guidelines. Larger zones indicate decreased bacterial growth with greater antibiotic susceptibility, whereas smaller zones show increased bacterial growth with less antibiotic susceptibility. If the antibiotic does not inhibit growth, there is no zone of inhibition—therefore, the bacteria are resistant.

Dilution Testing

Common dilution tests include broth dilution and agar dilution. In both tests, bacteria are placed onto multiple plates, tubes, or wells containing a specific concentration of antibiotic.[64] For example, the highest antibiotic concentration in a set of wells could be 4.0 ug/mL. The next well after

Figure 3 Broth Microdilution



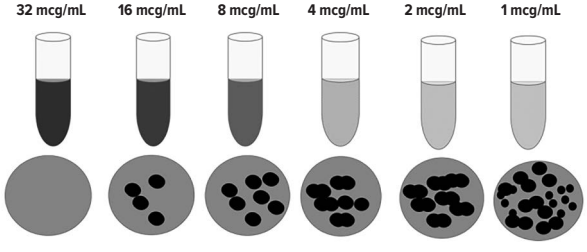
A quantified amount of microorganism is added to each well, with varying concentrations of eight antibiotics (A–H). Black circles indicate no bacterial growth. The minimum inhibitory concentration (MIC) is determined by identifying the lowest concentration that inhibits bacterial growth. The MIC for antibiotic A is 32 mcg/mL and for antibiotic C, 0.5 mcg/mL.

one dilution would have a concentration of 2.0 ug/mL, then 1.0 ug/mL after two dilutions, and 0.5 ug/mL after three dilutions. An example of broth microdilution is shown in Figure 3, and agar dilution is shown in Figure 4. Dilution test results can vary, and one-fold dilution error is common. For example, if the MIC is determined to be 1 ug/mL, the true MIC range could be from 0.5 to 2 ug/mL. This can be problematic if the range includes a susceptible and an intermediate MIC value.

Broth dilution consists of microdilution and macrodilution. Although they are similar, the methods differ primarily by the modality that is used to perform susceptibility testing (wells or tubes).[65] Broth microdilution is performed by placing different antibiotics with varying concentrations in wells that contain liquid media, as seen in Figure 3. Bacteria are added to each well, and the tray is incubated. The presence of bacterial growth is then identified through visual inspection of the plate. The MIC of the broth microdilution test is determined when no growth is observed with a particular antibiotic. An advantage of this test is that more than one antibiotic can be tested at a time. Disadvantages are that broth dilution is labor-intensive and time-consuming, and potential procedural errors may occur.

The agar dilution test is similar to the broth dilution test, the major difference being that it uses physical media as opposed to liquid media. Agar dilution is performed by placing standard concentrations of the organism on agar plates that vary in concentration of antibiotic, as shown in Figure 4.[64] The MIC is determined in the first test tube that demonstrates no growth of the organism. This test is commonly used when testing multiple bacteria against a particular antibiotic. Agar dilution is also labor-intensive and time-consuming, and it cannot test more than one antibiotic at a time.[65]

Figure 4 Agar Dilution



A quantified concentration of microorganism is on an agar plate. The minimum inhibitory concentration (MIC) is determined by identifying the lowest concentration of antibiotic that inhibits bacterial growth. MIC is measured in this example, where the antibiotic has a MIC of 32 mcg/mL.

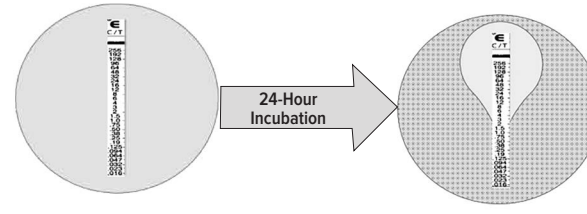
Combined Diffusion and Dilution Testing

The E-test is a quantitative test that combines diffusion and dilution techniques.[64] Instead of a disk, the test uses an E-test strip containing an antibiotic that diffuses across an agar plate containing bacteria. The strip has varying concentrations of antibiotic implanted along it (Figure 5). Unlike dilution testing, the differences in concentration are not one dilution apart; instead they are closer together, which can allow for a greater degree of precision. Once the plate has been incubated for 24 hours, the zone of inhibition creates an ellipse and intersects the strip on the plate. The point of intersection determines the MIC value. The E-test is advantageous because a stable gradient is used with a higher inoculum of bacteria, which enables the MIC values to be read more precisely. The disadvantages are that the strips can only test one antibiotic, and the test can be costly and is labor-intensive.

Automated Systems

Automated systems have the advantage of being less labor-intensive and of enabling quicker reporting of results.[66] These systems primarily use dilution principles to determine antibiotic susceptibility. Fully automatic systems will introduce bacteria to a panel, incubate them, then read and interpret the susceptibility results. Automated tests determine MIC through the use

Figure 5 E-test



The microorganism is streaked onto the agar plate and an E-test strip is placed on top. This agar plate is incubated for 24 hours whereupon an ellipse forms around the strip. The intersection point is known as the minimum inhibitory concentration (MIC), and for the antibiotic in this example, the MIC is 2 mcg/mL.

of algorithms and allow rules that predict resistance to other antibiotics. Also, these systems can save results to be used in the creation of antibiograms along with other reports. The FDA sets standards for the approval of automated systems, which include a rate of false resistance of less than 3% and a false susceptibility of less than 1.5% on the lower end of the 95% confidence interval (CI) and less than 7.5% on the upper end of the 95% CI.[67] They must also agree, within one dilution, in reference breakpoint testing more than 90% of the time. A variety of automated systems are available, including VITEK 2, Microscan Walkaway plus, Phoenix, and Sensititre. Each system has a variety of panels that test different bacteria and different drug combinations. Although errors can occur, this happens at the drug–bacteria combination level. If errors are expected, results should be confirmed with manual testing. Each system is constantly updated and may perform differently depending on the drug–bacteria combination, making it difficult to recommend one system over another.

In Vitro Versus *In Vivo* Results

Antibiotics may fail to achieve a cure in the clinical setting (*in vivo*) despite showing susceptibility in the laboratory (*in vitro*). This can occur as a result of resistance mechanisms, toxin production, or the pharmacokinetic and pharmaco dynamic properties of antibiotics. This article focuses on resistance mechanisms, and readers can refer to reviews of toxins, pharmacokinetics, and pharmacodynamics for further information.[68,69] Resistance can develop while a patient is on antibiotic therapy; this has been well documented with clindamycin.[70] The clindamycin D-test, which uses disk-diffusion principles, can help detect inducible clindamycin resistance in *S. aureus* and β-hemolytic streptococci. To perform the test, a disk containing clindamycin and erythromycin are placed on the same plate and the plates are incubated. If the zone of inhibition appears as a circle, there is no inducible resistance; if it appears as a "D," there is inducible resistance.

As new resistance mechanisms develop, laboratories need to develop new ways to detect resistance. One example of this is the detection of extended-spectrum beta lactamase (ESBL) and/or AmpC beta-lactamase.[71,72] ESBLs are enzymes that break down many beta-lactam antibiotics, rendering them ineffective. They are more likely to be present in gram-negative bacteria that are associated with health care-related infections. To detect ESBL, screening is performed by testing certain antibiotics like cefotaxime and then adding clavulanic acid. If ESBL screening is performed using broth microdilution, the MIC is compared between cefotaxime and cefotaxime plus clavulanic acid. If there is a threefold dilution change in MIC when clavulanic acid is added, ESBL is present. For example, if the MIC is 2 ug/mL with cefotaxime alone and decreases to 0.5 ug/mL when clavulanic acid is added, this suggests that ESBL is present. The interpretation of ESBL can be further complicated by the presence of another beta-lactamase, AmpC. This can cause false-negative screening for ESBL, but it can be detected via the E-test. New resistance mechanisms are likely to be identified in the future, which will provide new challenges in achieving clinical cure.

CONCLUSION

This article reviewed the necessary information for interpreting culture results including timing of cultures, common culture sites, interpreting the Gram stain, rapid diagnostic tests, conventional antibiotic susceptibility testing, and automated testing. Ideally, cultures should be drawn prior to the patient receiving antibiotics, and antibiotics should be received within an hour. Cultures should be obtained in a way that optimizes specificity and sensitivity, although clinicians should be aware of the diagnostic tests used at their institutions and their limitations. Both rapid diagnostic tests and conventional susceptibility testing can be used to select the most appropriate antibiotic. Using this guide can assist pharmacists and clinicians in improving patient safety.

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